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In vitro evaluation of the antioxidant activities of carbohydrates

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ABSTRACT

In the current study, we evaluated the antioxidant activities of highly purified monosaccharides, oligosaccharides and complex carbohydrates using six in vitro antioxidant assays, including oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), β-carotene bleaching assay, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging and superoxide radicals scavenging methods. The results suggested that monosaccharides and oligosaccharides scarcely exhibited antioxidant activities in vitro. For example in the β-carotene bleaching assay the inhibitory effects of monosaccharides at 1 mg/mL were between 0.24% to 2.25% although galacturonic acid demonstrated some inhibitory effects (8.59%). Significant lower antioxidant activities were observed for complex carbohydrates compared to ascorbic acid and BHT (e.g. ferric reducing antioxidant power by the FRAP assay), > 1800 μmol Fe (II)/g were observed for ascorbic acid and BHT compared to only 10–60 μmol Fe (II)/g for complex carbohydrates, and 1.60 and 6.43 μmol Fe (II)/g for mono and oligosaccharides. The observed antioxidant activities for complex carbohydrates are correlated with the presence of phenolic and/or protein components. The findings from the current study indicate that it is necessary to re-evaluate the antioxidant activities claimed for complex carbohydrates in the literature as many of them did not consider the contribution of other minor components such as phenolics and proteins.

1. Introduction

Oxidative stress refers to the imbalance of oxidation and reduction in vivo. It leads to the production of oxidation intermediates which could cause base damage as well as strand breaks in DNA, hence become a serious threat to human health (Seifried, Anderson, Fisher, & Milner, 2007). Antioxidant components from food have been considered beneficial to human health because they can slow the effects of oxidative stress (Cook & Samman, 1996). Antioxidants are also known to reduce the risk of Alzheimer's disease, cataracts, some types of cancer and cardiovascular diseases (Halliwell, Aeschbach, Löliger, & Aruoma, 1995; Oak, El Bedoui, & Schini-Kerth, 2005), improve human immunity and suppress inflammatory reactions (Yao et al., 2004). Thus, accurately evaluation of the activities of antioxidants of food components is important.

Many compounds from plant extracts exhibited antioxidant activities, such as pigments (fucoxanthin, anthocyanins, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.) (Heo, Park, Lee, & Jeon, 2005; Lai et al., 2013; Soong, & Barlow, 2004; Van Acker et al., 1996). Numerous studies have reported that some selected polysaccharides have various biological activities, including antitumor activities, immunomodulatory effects and antiviral activities (Lv, Cheng, Zheng, Li, & Zhai, 2014; Ren et al., 2015; Xu et al., 2009; Zheng, Dong, Chen, Cong, & Ding, 2015). Some recent studies also consider polysaccharides as sources of novel potential antioxidants (Chen, Ma, Liu, Liao, & Zhao, 2012; Ferreira et al., 2014; Fu, Chen, Dong, Zhang, & Zhang, 2010; Petera et al., 2015; Ramarathnam, Osawa, Ochi, & Kawakishi, 1995). However, most of the studies on antioxidant activities of polysaccharides usually indicated they were mixed with some minor components, such as proteins and some low molecular weight substances (Chen, Zhang, Qu, & Xie, 2008; Wang, Zhao, Andrae-Marobela, Okatch, & Xiao, 2013; Wang, Wang, Li, & Zhao, 2001). For example, Siu, Chen, and Wu (2014) suggested that the antioxidant activities of crude polysaccharide from mushrooms were mainly attributed to the phenolic and protein components, while Wang et al. (2013) proved that tea polyphenols were the major antioxidants in the

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crude tea polysaccharides. There were also reports demonstrated that the antioxidant activities of polysaccharides could be significantly affected by modifications, such as sulfation and/or acetylation (Chen et al., 2014; Delattre et al., 2015; Jin, Wang, Huang, Lu, & Wang, 2014; Qi et al., 2005; Wang et al., 2010; Wang, Zhang, Zhang, & Li, 2008). On the contrary, the antioxidant activities of purified polysaccharides and their fractions have been reported to be much weaker (Wang & Luo, 2007). These variations on antioxidant activities of polysaccharide raised our curiosity. Does carbohydrate have antioxidant activities in vitro? Until present, little information could be found in the literature on the antioxidant activity of carbohydrates.

Carbohydrate is known as polyhydroxy aldehydes and ketones, or substances that can be hydrolyzed to yield polyhydroxy aldehydes and ketones. It is covalently linked by glycosidic linkages, which are formed by condensation reaction between the glycosyl moiety of hemiacetal or hemiketal, together with the hydroxyl group of another sugar unit (Cui, 2005). Hence, there are few aldehyde or ketone group in carbohydrate solution; based on these structural characteristics we could hypothesis that pure carbohydrates do not have strong antioxidant activities. To answer and confirm the question: the objectives of current study were to systematically evaluate the antioxidant activity of monosaccharide, oligosaccharide and some complex carbohydrate. Physical-chemical and compositions of complex carbohydrates studied were also determined to elucidate if other components contributed to the antioxidant activities of complex carbohydrates.

2. Materials and methods

2.1. Materials and chemicals

D - (+) - glucose, sucrose, lipopolysaccharide, polygalacturonic acid, pectin, xylan, arabinogalactan and D - (-) galacturonic acid were purchased from Sigma Chemical Co. (St. Louis, MO USA). D - (+) - galactose, D-mannose, xylose, D-lactose, raffinose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, pullulan and D-glucuronic acid were from J&K Co. (Shanghai, China). D-(-) arabinose was the product of Solarbio Co. (Shanghai, China). Galactomannan, arabinoxylan and glucomannan were obtained from Megazyme Co. (Wicklow, Ireland).

Trolox, 2, 2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, 2, 4, 6-Tris (2-pyridyl)-s-triazine (TPTZ), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and Coomassie Brilliant Blue G-250 were obtained from Sigma Chemical Co. (St. Louis, MO USA). Linoleic acid, β-carotene and pyrogallic acid were from Aladdin Co. (Shanghai, China). Bovine serum albumin (BSA) was from Solarbio Co. (Beijing, China). Gallic acid was purchased from J&K Co. (Shanghai, China). All other chemicals were of analytical reagent grade. All aqueous solutions were prepared using freshly double distilled water.

2.2. Antioxidant activities of monosaccharide

The antioxidant activities of different monosaccharides were evaluated by oxygen radical absorbance capacity, ferric reducing antioxidant power, β-carotene bleaching assay, DPPH radical scavenging, hydroxyl radical scavenging and superoxide radicals scavenging assessments. Each test was performed in triplicates.

2.2.1. Oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity was measured using a fluorescence microplate reader (Thermo Fisher Scientific, Thermo Electron Co., Waltham, MA, USA) described by previous report (Folch-Cano, Jullian, Speisky, & Olea-Azar, 2010), with slight modifications. The ORAC method is based on trolox as an antioxidant standard and 2, 2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) as a peroxyl generator. The peroxyl radical scavenging was measured using fluorescein as fluorescent probe. The degree of change in fluorescence intensity reflects the extent of free radical damage. Antioxidant can inhibit the fluorescence decay caused by free radicals, reflecting its ability to inhibit free radicals (Barahona et al., 2014). The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4). Sample, standard and phosphate buffer (60 μL, 75 mM, pH 7.4) were placed in the wells of the microplate. Then fluorescein solution (40 μL, 1.75 μM) was added. The mixture was pre-incubated for 15 min at 37 °C, before adding the AAPH solution (100 μL, 10 mM). The microplate was shaken automatically for 30 s and then was immediately placed in the reader. The fluorescence (excitation wave length of 460 nm, emission wave length of 515 nm) was recorded every 2 min for 120 min.

The ORAC values were calculated as the area under the curve (AUC) and the inhibition capacity was expressed as Trolox equivalents (μmol Trolox/g).

2.2.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed as described previously (Xu & Chang, 2007) with some modifications. Antioxidant donates an electron to TPTZ-Fe (III) complex, which could be reduced to TPTZ-Fe (II) form. TPTZ-Fe (II) has an intensive blue color and can be monitored at 593 nm (Benzie & Strain, 1996). Briefly, The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3$ in a volume ratio of 10:1:1. Sample solution (200 μL) was mixed with 1.8 mL working FRAP reagent, and incubated at 37 °C for 30 min The absorbance was measured at 593 nm. FRAP value was calculated using the concentration of $FeSO_4 \cdot 7H_2O$ equivalent. Ascorbic acid and BHT were used as positive controls. The reducing capacity was expressed as μmol Fe (II)/g.

2.2.3. β-carotene bleaching assay

Antioxidant activity was carried out according to the β-carotene bleaching assay (Chen et al., 2014). Linoleic acid becomes a free radical with a hydrogen atom abstracted. The radical formed then attacks β-carotene molecule and the compound loses its characteristic orange color. Antioxidant can neutralize the linoleate free radical and thus inhibit the oxidation of linoleic acid (Deba, Xuan, Yasuda, & Tawata, 2008). β-carotene (0.5 mg) was dissolved in 1 mL of chloroform. Twenty five μL linoleic acid and 200 mL Tween 20 were added. The chloroform was evaporated under nitrogen at 45 °C, then 100 mL distilled water was added and shook vigorously. Three hundred and fift μL of sample and 2.5 mL of the β-carotene-linoleic acid emulsion were mixed into test tube. The absorbance was immediately measured at 490 nm. The reaction mixture was incubated at 50 °C for 2 h and the absorbance was measured again. The ascorbic acid and butyl hydroxyl toluene (BHT) were used as a positive control.

2.2.4. DPPH radical scavenging activity

Relatively stable DPPH radical has been widely used to evaluate the free radical scavenging capacities of antioxidants. Antioxidant transfer hydrogen atoms to DPPH·, leading to non-radical form DPPH-H (Li, Li, & Zhou, 2007). The antioxidant activity of a substance can be expressed as its ability in scavenging the DPPH radical. The scavenging effect of the sample on DPPH radical was measured according to the method as previously described (Wang et al., 2013). Sample solutions (1.0 mL) with variable concentrations were added with 2.0 mL DPPH (0.2 mM in ethanol). The mixture was shaken and kept at room temperature in the dark. The absorbance was measured at 517 nm 30 min later. The control was done with ethanol instead of DPPH, while ethanol was used as the blank. The DPPH radical scavenging activity (%) was calculated according to the following equation: